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Please replace the paragraph beginning at page 9, line 4, with the following rewritten paragraph:

A3

– Figure 15 illustrates a block diagram of an instrument in which the test piece is movable in X and Y directions; –

/

Please replace the paragraph beginning at page 9, line 6, with the following rewritten paragraph:

A4

– Figure 16 is a perspective view illustrating certain of the components of Figure 15 including a laser subsystem, a X-Y subsystem, an optical subsystem and the light collection device; –

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Please replace the paragraph beginning at page 9, line 9, with the following rewritten paragraph:

A5

– Figure 17 is an exploded view of the components of Figure 16; –

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Please replace the paragraph beginning at page 9, line 10, with the following rewritten paragraph:

A6

– Figure 18 illustrates some of the components of Figure 17 assembled together but with laser subsystem and Z movement components being shown in exploded view; –

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Please replace the paragraph beginning at page 9, line 12, with the following rewritten paragraph:

A7

– Figure 19 illustrates some of the components of Figure 16 and diagrammatically depicts the light beam input from the laser subsystem and the light received by the optical subsystem to be input into the light collection device; –

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Please replace the paragraph beginning at page 9, line 15, with the following rewritten paragraph:

A8

– Figure 20 illustrates a front panel of the instrument of Figure 15 including controls related to controlling image data and indicators related to information associated with a number of subspots for one spot on the test piece; –

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Please replace the paragraph beginning at page 9, line 18, with the following rewritten paragraph:

A9

– Figure 21 is a graph illustrating a histogram of the number of pixels at different grey levels; –

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Please replace the paragraph beginning at page 9, line 20, with the following rewritten paragraph:

A10

– Figure 22 is a flow diagram related to the providing of instrument settings and positions; –

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Please replace the paragraph beginning at page 9, line 22, with the following rewritten paragraph:

A11

– Figure 23 is a flow diagram related to main steps conducted in testing one or more subspots of one or more spots found on a test piece; –

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Please replace the paragraph beginning at page 9, line 24, with the following rewritten paragraph:

A12

– Figure 24 is a flow diagram identifying certain major steps involved with processing of image data using light intensity; and –

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Please replace the paragraph beginning at page 9, line 26, with the following rewritten paragraph:

A13

– Figure 25 is a flow diagram identifying certain major steps related to image analysis using size or appearance. –

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Please replace the paragraph beginning at page 22, line 25, with the following rewritten paragraph:

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A14

– Mass enhancement labels can play a central role in the practice of the enumeration method at high sensitivities. Figure 13 illustrates, proportionally, the aspect ratio or relative height:width:breadth of various size materials that may be used as signal generators. As is diagramed in this figure, organisms at the cellular scale generate very significant signal without amplification within the system. In comparison, the thin attachment layer represented along the bottom of the reading zone surface creates a clearly distinguishable signal with the current OTER format. The signals generated by mass contained in the much larger objects used as labels significantly improve sensitivity. –

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Please replace the paragraph beginning at page 23, line 15, with the following rewritten paragraph:

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A15

– Because the change in polarization state detected by ellipsometry is caused by two distinct factors (absolute mass and refractive index) the use of a high refractive index material as the mass enhancement label effectively increases the apparent mass detected by the ellipsometer, thus, further amplifying the signal from the binding event. –

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Please replace the paragraph beginning at page 26, line 9, with the following rewritten paragraph:

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A16

– With respect to analyzing a test piece, an embodiment of an instrument for obtaining data and making determinations using light scattering principles is illustrated in Figure 14. Generally, and referring to Figure 14, a prepared test piece is secured to the sample stage and manually positioned such that the center of a test spot is aligned with the center of the objective lens. The test piece may be prepared to contain multiple test spots, therefore, to begin the test spot designated as 1, or first, is centered. Using the sample stage's translational capabilities (the detector could be alternatively or additionally moved, manually and/or automatically), the detector is manually focused on the scattering particles. Next, the image produced by the light scattering is collected and saved. Finally, the sample stage is

A16

translated to two alternate locations, one each to the left and right of center, and image acquisition repeated at each location. Each generally herein-described step in the detection process may be repeated for any number of test spots contained on a test piece. –

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Please replace the paragraph beginning at page 26, line 21, with the following rewritten paragraph:

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A17

– The instrument employed for the enumeration methodology disclosed herein consists of three defining modules: a sample stage, an optical signal format corresponding to the immobilized analyte complex, and a means for data collection and analysis. Each module is adapted for independent translation on at least two axes, thereby facilitating optimal optical effect, alignment and focus. The instrument and its modules, *in toto*, are fixed and stationary in relation to one another by standard attachment means to, for example, a solid, planar, horizontal platform. More specifically, as shown in Figure 14, the enumerator 100 is comprised of a means for data collection and analysis 85 consisting essentially of a computer 80 and video display terminal 60 functionally combined with a sample stage 10 and optical signal format consisting essentially of a signal carrier 40 and a signal detector 25 configured such that when a signal generator, such as a light scattering label, is irradiated, it is able to be detected by the enumerator 100. –

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Please replace the paragraph beginning at page 28, line 25, with the following rewritten paragraph:

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A18

– With reference to Figure 15, a block diagram of a particular instrument 200 for determining whether a substance of interest, such as a particular or target analyte, is present with a sample under test is illustrated. The sample under test, in this embodiment, is movable in controlled X and Y directions using a X-Y subsystem 204. The test piece subsystem 208 is held to the X-Y subsystem 204 and moves therewith. The test piece subsystem 208 preferably includes a test piece having a number of test spots that contain one or more samples that are to be tested for one or more substances of interest. In a preferred embodiment, each of the test spots has a number of test subspots. Each of the test subspots

A19  
cont'd

may have only one substance of interest, although one or more of the subspots may have a different substance of interest, which, in one embodiment, is to be detected (if present) and not detected (if not present). In another embodiment in which there is an indirect assay format, a detection is made when the substance of interest is not present and a detection is not made when the substance of interest is present. --

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Please replace the paragraph beginning at page 33, line 7, with the following rewritten paragraph:

A19

-- Referring to Figures 16-18, greater structural and operational details are described in conjunction with an embodiment of the X-Y subsystem 204, laser subsystem 212, optical subsystem 216 (Figure 15) and light collection device 220. In this embodiment, the X-Y subsystem 204 includes a X subsystem 250 used in enabling movement in the X direction. The X subsystem 250 includes a frame 254 and a X-rod or track 258. The X-rod 258 is joined to a X-connector 262 that communicates with the output of the X servomotor. The rotational output of the X servomotor, which is applied to the X-rod 258 through the X-connector 262 causes controlled translational or linear movement of the X subsystem 250 in the X direction. The X-Y subsystem 204 also includes a Y subsystem 266 comprising a Y-frame 270, a Y-rod or track 274 and a Y-connector 278. The output from the Y servomotor communicates with the Y-rod 274 through the Y-connector 278 in connection with providing relative movement between the Y-rod 274 and the Y-frame 270 in order to enable movement of the Y subsystem 266 in the Y direction. The X subsystem 250 and the Y subsystem 266 are joined together using a X-Y plate 282 that is illustrated in Figure 17.

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Please replace the paragraph beginning at page 33, line 21, with the following rewritten paragraph:

A20

-- The test piece subsystem 208 is joined to the X-Y subsystem 204 by, in this embodiment, portions of the Y-frame 270. The test piece subsystem 208 can include a test piece base 286, a test piece side 290 and a test piece front 294. As depicted in Figure 16,

A20

each of these test piece parts can be joined together and the test piece 300 is held using these three test piece parts. The test piece base 286 is joined to the Y-frame 270 of the Y subsystem 266. Consequently, movement in the X direction and/or Y direction using the X-Y subsystem 204 causes movement of the test piece subsystem 208 including the test piece 300 having one or more samples that are to be analyzed by the instrument 200. –

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Please replace the paragraph beginning at page 34, line 1, with the following rewritten paragraph:

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A21

– With regard to the laser subsystem 212, it is also joined to the base plate 304 to which the X-Y subsystem 204 is connected. Referring to Figure 17, in one embodiment, the laser subsystem 212 includes the laser device 310 that is joined to a laser holder 314 which can be in the form of a C-clamp configuration having a cylindrical bore that receives the laser device 310. The laser holder 314 can have at least one slot 334. The laser holder 314 is joined to a laser support 318 having a foot portion 322 with a slit 326. The laser holder 314 can be held at a selected angular position to the laser support 318. Depending upon the location of the laser holder 314 relative to the slot 334, a selected, desired angle of the light beam output from the laser device 310 can be provided. The angle of the light beam is relative to the surface of the test piece 300. The laser support 318 is also joined to the base plate 304 and can be laterally, selectively positioned by joining the foot portion 322 to the base plate 304 at a selected part of the slit 326. Hence, the laser device 310 can be controllably positioned in a substantially lateral direction relative to the test piece subsystem 208 including the test piece 300 itself to obtain desired location of the laser light or light beam from the laser device 310 on the test piece 300. –

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Please replace the paragraph beginning at page 34, line 17, with the following rewritten paragraph:

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A22

– Referring to Figure 18, a Z-rod or track 344 is joined to the Z-frame 340. The Z subsystem 232 can be manually movable whereby the Z-frame 340 moves relative to the Z-

A22

rod 344 to adjust its position in the Z direction relative to the test piece 300. In another embodiment, the Z subsystem 232 can be automatically controlled using the control 230. –

Please replace the paragraph beginning at page 34, line 21, with the following rewritten paragraph:

A23

– Referring again to Figure 17, a video objective 360 is illustrated that can be held by a lens cell holder 350 (Figure 16). The lens cell holder 350 can also be a C-clamp configuration with a cylindrical bore that holds the video objective of the optical subsystem 216 used in receiving scattered light from the test piece 300. The embodiment of Figure 17 also depicts a Z-plate 364 that is used to provide greater controlled movement in the Z direction. Attached to the Z-plate is a plate 368 to which the light collection device 220, such as the digital camera, can be joined in connection with achieving desired movement in the Z direction relative to the test piece 300. –

Please replace the paragraph beginning at page 35, line 1, with the following rewritten paragraph:

A24

– With reference to Figure 19, a schematic representation is provided showing the light beam being output from the laser subsystem 212 to one of the test spots 302 on the test sample 300. The light beam is directed unobstructed to the subject test spot and from portions thereof, scattered light results. The scattered light is received by the optical subsystem 216 including its video objective 360. From there, the scattered light is directed to the light collection device 220 for subsequent processing. As can be understood, the laser subsystem 212 can be located at a desired angle relative to the test spots 302 by initial selective adjustment using the slot 334. The lens cell holder 350 can also be adjusted. In such a case, the adjustment is essentially linear in the Z direction. After completion of any such adjustment, the light beam is able to controllably strike or contact each of a selected or desired one of the spots 302. In particular, neither the optical subsystem 216 nor the light collection device 220 cause an obstruction to the light beam as it is directed to a particular spot 302 on the test piece 300. This unobstructed path remains as the test piece 300 is moved



A24

in X and Y directions during the relative movement between the test piece 300 and the light beam, as part of the testing of the test spots 302 in connection with determining whether a particular analyte or other substance of interest is present with one or more of the test spots 302. –

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Please replace the paragraph beginning at page 35, line 18, with the following rewritten paragraph:

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A25

– In connection with the desired testing, the next description relates to certain controls and indicators that can be provided in achieving acceptable test results. Figure 21 conveniently depicts a conglomeration of a number of software generated computer screens that relate to controllable functions useful in determining whether a particular substance of interest is present with the sample under test. Regarding the light collection device 220, such as a digital camera, each of its gain and its integration time (shutter speed) can be separately regulated. In one embodiment, a mouse or other input device to the computer of the control 230 is controlled by the operator or user in connection with increasing or decreasing one or both of the digital camera gain and shutter speed. Generally, the magnitudes of control for each of these two parameters of the light collection device 220 is determined by empirical information gathered or known by the operator. For example, in cases in which the substance of interest under test has been previously tested for, the information obtained concerning gain and shutter speed that achieved accurate or acceptable results in the previous test may be relied upon to determine whether that same substance of interest is present with the current sample being tested. The control of each of the gain and shutter speed is used to provide light or image data that enhances the acceptability or accuracy of the ultimate determination related to the detection and/or measurement of the substance of interest, if present. In one embodiment, the parameters of the light collection device 220 can be adjusted during processing/analyzing procedures in determining whether a substance of interest is present with the sample under test. The parameters can be initially provided and utilized during the testing and, subsequently, based on obtained information and processing/analysis that was completed, one or both of these parameters could be adjusted

A25

to better or enhance the image data being obtained. It is preferred that any such subsequent adjustment that might occur during testing be implemented automatically, which automatic determination can rely on one or more of a number of factors related to the intensity of the light being received. –

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Please replace the paragraph beginning at page 36, line 15, with the following rewritten paragraph:

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A26

– The control panel 240 of Figure 21 identifies a look up table (LUT) function or application, which can be selectively activated or de-activated by the operator using an input device, such as a "button" that can be controlled by touch, mouse manipulation or other suitable selection. When activated, the selected LUT application enhances the brightness and contrast of images (image data or other information) by modifying the dynamic intensity of image data or regions thereof that have relatively poor contrast. A LUT transformation converts input grey level values obtained by the light collection device 220 as a function of a sample under test into other grey level values that constitute a transformed image having transformed image data. The LUT applications that can result in such a transformation are essentially mathematical tools implemented by software that are executed by the computer of the control 230. There are a number of predetermined LUT applications for selection in connection with enhancing the brightness and contrast of the image data. These LUT applications can include the following: linear, log, exponential, square, square root, power  $X$  and power  $1/X$ . One or more of these mathematical tools, or other similar tools, is selectable by the operator to achieve the desired function. Typically, if the LUT application is activated, only one of them is utilized for a particular test sample. As also seen in this illustration of the control panel 240, the operator can select a  $X$  value that is used when the LUT application is power  $X$  or power  $1/X$ . The selected power is used with a pixel value and, in particular, mathematically manipulates or acts on that pixel value in conjunction with changing the dynamic range of the pixel values. The pixel refers to the smallest or finest dimension of the light collection device 220, such as the resolution of the digital camera that can be defined as including an array of pixels. In one embodiment, the pixel values can be

A26  
cont'd

in the range of 0-255, with a zero pixel value referring essentially to a black pixel and the pixel value 255 essentially referring to a completely white pixel. For example, the power X application is used to make particles, when present, appear bright on a uniformly black background. The value of X in this embodiment is about 2-3, such as 2.80. A mathematical calculation involves raising the pixel value to the 2.8 power in this example. For a pixel value of 100, the mathematical calculation involves  $100^{2.8}$ . In accordance with this example, after the mathematical calculation relatively more pixels would be assigned a pixel value of 255 and other pixels would be assigned, on a relative basis, pixel values less than 255. –

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Please replace the paragraph beginning at page 37, line 16, with the following rewritten paragraph:

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A27

– A thresholding control function is also identified by the control panel 240 of Figure 20. Thresholding involves segmenting image data into two regions, namely, a particle (or object intended to be indicative of the target analyte) region and a background region. When implementing a thresholding process, all pixels can be set to a binary 1 when their pixel values equal or exceed a grey level value that can be defined as the lower limit threshold limit, while all other pixels having pixel values less than the lower limit threshold limit can be set to a binary 0. Alternatively, the pixels equal to or exceeding the threshold can be set to a binary 0 and those below can be set to a binary 1. In one embodiment, the lower limit threshold value, which is at the lower end of the thresholding interval, is determined using a histogram analysis. The histogram provides the frequency of a given distribution of pixel values for the particular collected image data. For example, if 100 pixels in the image data have a pixel value of 20, then the frequency for the pixel value of 20 is 100. Referring to Figure 21, a representative histogram is illustrated for a grey level range of 0-255. The numbers of pixels are noted for different pixel values along the grey scale range. For each pixel value, an analysis is conducted using the number of pixels having that pixel value. A determination of the minimum threshold value or lower limit is determined by finding the maximum frequency peak for a given distribution of pixel values. Based on the determined minimum threshold value, any pixel value that is less than the minimum threshold value is

A27  
cont'd

assigned a binary 0 and those greater than or equal to the minimum threshold value are assigned a binary 1. As a result, those assigned a binary 0 are removed from any further consideration or analysis in connection with determining particles or objects evidencing the substance of interest. In one embodiment, a maximum value or upper limit can be defined and input to control which pixel values are to be used in the subsequent determinations. The maximum value or upper limit is typically operator selected and manually input using the mouse or other computer input device. In one embodiment, similar to gain or shutter speed settings, the maximum value is found empirically or by "trial and error." Previous determinations of the upper limit for a particular substance of interest can be relied upon in arriving at the current maximum value. Referring to Figure 20, the lower value and the upper value indicators refer to the minimum and maximum threshold values, respectively.

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Please replace the paragraph beginning at page 40, line 28, with the following rewritten paragraph:

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A28

— The control panel 240 of Figure 20 also depicts operator control over interpolation of pixel values. An image data indicator related function is provided by means of the subsample indicator. According to this function, a correspondence or correlation is provided between the pixels associated with the digital camera and the pixels on the computer screen or display 234. When causing a display depicting the image data of the digital camera pixels, it may be desirable to have a reduced image size whereby a number of digital camera pixels corresponds to one point or pixel on the computer screen. For example, a subsample value of three means that the computer screen has one display point or one display pixel that corresponds to three digital camera pixels. —

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Please replace the paragraph beginning at page 42, line 3, with the following rewritten paragraph:

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A29

— When using the instrument 200, particularly the laser subsystem 212, the light beam covers and focuses on the entire spot so light strikes or is received by all test subspots of the

A29  
Cont'd

test spot under test at the same time. Each subspot has a correlated or corresponding number of digital camera pixels. Thus, certain of predetermined pixels can be processed and analyzed for each particular subspot. Related to this arrangement is that different samples being tested could be provided on different subspots. That is, a first substance of interest might be tested using subspot one and a second substance of interest might be tested using subspot two. In determining whether one or more substances of interest is present with a test spot, each of the subspots can be separately processed and analyzed. As part of the enumeration method, the particles or objects that are counted after the image processing and analysis are completed can be separately counted for each subspot. In the case in which the same substance of interest is being tested for on all subspots of a particular test spot, after all the subspots have been analyzed and the particles counted for each, the total number of particles can be counted based on the counts made for each of the subspots. When each subspot or any number of subspots, which are less than all of the subspots for a particular test spot, have a first substance of interest, while one or more other subspots have at least a second substance of interest, separate particle counts are made for each such subspot or combination of subspots in determining whether a substance of interest is present. With respect to processing and analyzing subspots, in one embodiment, a substantially serpentine path is utilized when conducting such processing and analysis, particularly in an embodiment where there is a substantial number of subspots, such as the embodiment with the magnification of 10x. According to the serpentine path, the subspots of row 1 (0, 1, 2, 3) are separately analyzed in that order and then the subspots of row 2 (9, 8, 7, 6, 5, 4) are analyzed beginning with subspot 4. Then, for row 3 of subspots, the analysis is conducted right-to-left based on the representation in Figure 21 and so forth until all subspots in row 12 have been processed and analyzed. —

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Please replace the paragraph beginning at page 43, line 1, with the following rewritten paragraph:

A30

— With reference to the flow diagrams of Figures 22-25, the operation of the instrument 200 is further described. Referring to Figure 22, as part of testing one or more

A30

samples with a test piece 300, the operator or user initially establishes settings and/or positions associated with the instrument 200. At block 500, the optical subsystem 216, or one or more elements thereof, is located at a desired position in the Z direction. In the embodiment that includes Figure 18, the objective tube lens can be positioned in the Z direction so that the optical subsystem 216 is desirably located relative to the test piece subsystem 208. According to one setup process, the optical subsystem 216 is located in an acceptable position and can remain in that position for any number of test piece subsystems 208 and samples being tested. –

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Please replace the paragraph beginning at page 43, line 10, with the following rewritten paragraph:

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A31

– At block 504, steps can be taken to position the laser subsystem 212 so that its light beam output contacts or strikes the particular test spot 302 having the sample being tested without obstruction. Such positioning of the light beam can include adjustments related to lateral position and/or an angular position using the parts of Figure 17. Like the setup for the optical subsystem 216, once it is finished for one sample being tested or one particular test piece subsystem 208, it may be that the laser subsystem 212 can remain in that position for any one of a number of samples being tested. The position of the laser subsystem 212 that affects the location of its light beam output can be automatically controlled, as well as manually controlled, just as can the location of the optical subsystem 216. –

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Please replace the paragraph beginning at page 44, line 1, with the following rewritten paragraph:

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A32

– At block 516, positioning of the test piece subsystem 208 having the test spots 302 is accomplished. In the case in which the test subspot to be tested is not properly located, the test piece subsystem 208 is moved using, for example, the X-Y subsystem 204 by means of the hardware or parts illustrated in Figs. 16-18. In one embodiment, the indicator on the control panel 240 depicting the test spots available for testing for a particular test piece 300

A32  
cont'd

having 12 test spots can be used to properly position the X-Y subsystem 204. Selecting a particular test spot using an input and the indicator on the control panel 240 can cause appropriate movement of the X-Y subsystem 204 so that there is proper alignment between the light beam and the selected test spot. –

Please replace the paragraph beginning at page 44, line 26, with the following rewritten paragraph:

A33

– With reference to Figure 24, main procedures or precesses available for processing the image data are illustrated. At block 540, the obtained image information/data is available for processing in the form of electrical signals and which information or data can be temporarily stored for processing using software and the algorithms that are executable using such software. At block 544, one or more look up tables (LUTs) can be accessed for manipulating the image data to enhance its brightness and/or contrast. That is, the image data obtained can be processed to provide a better representation thereof, such as desirably affecting the dynamic range of the obtained image data. In one embodiment, the available applications of LUTs include power  $X$  and power  $1/X$ . When one of these application is to be used, at block 548 a value of  $X$  is input that is based on a desired or optimum contrasting or enhancement of the input image data. –

Please replace the paragraph beginning at page 45, line 26, with the following rewritten paragraph:

A34

– In addition to the availability for selection of image processing procedures, Figure 23 identifies, at block 570 further procedures or series of steps that can be conducted as part of data image analyses. Referring to Figure 25, such analyses can include one or more morphology procedures at block 580. In one embodiment, the morphology software can analyze the results of the image data after thresholding. One or more related but different morphology applications can be invoked related to the appearance or size of such image data. The morphology application can desirably manipulate the data (e.g. dilate and/or close

A34  
cont'd

functions) to better prepare it for more accurate counting of particles or objects when present that are indicative of the substance of interest. –

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Please replace the paragraph beginning at page 46, line 17, with the following rewritten paragraph:

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A35

– When one or more of these processes are completed and the determination is then to be made regarding the presence of the substance of interest based on the number of particles, a return is made to Figure 23. In one embodiment, at block 600, the computer display or screen 234 can illustrate the result(s) of the processing and analysis that was conducted using the one or more procedures of Figures 24 and 25. Such results can include the number of particles that remain for counting or the counted number of particles that would be used in determining information related to the presence of the substance of interest. Regardless of whether or not such information is displayed, at block 604 of Figure 23, based on the image data related to the particles that remain, the relevant software is used to count such particles or objects for the current subspot being tested. At decision block 608, a check is made regarding whether another subspot is to be tested in connection with determining the presence of the particular substance of interest. If there is one or more such subspots, at block 612, the next subspot  $m$  of the current spot  $n$  is next to be used in obtaining light information or image data therefrom. In that regard, the testing is repeated including a return to the series of steps associated with block 520. On the other hand, if all particles have been counted for a particular substance of interest, at block 616, the number of particles that have been counted for one or more subspots and/or spots being used to determine whether the substance of interest is present for a particular sample, is stored or saved to computer memory. If there is another sample to be tested, then at block 620, this further sample can be tested. In one embodiment, this next sample may be such that the previous instrument 200 set up need not be changed. If there is a need to change the instrument 200 setup, one or more of the procedures identified by the blocks of Figure 22 can be employed before conducting the testing outlined by Figure 23. –

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